IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Dean L. Engelhardt et al.	
Serial No.	08/479,997	Group Art Unit: 1809
Filed:	June 7, 1995	Examiner: Scott Houtteman
Title:	A PHOSPHATE MOIETY LABELED NUCLEOTIDE, AND AN OLIGO- OR POLYNUCLEOTIDE, AND OTHER COMPOSITIONS COMPRISING SUCH PHOSPHATE MOIETY LABELED NUCLEOTIDES	

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Honorable Commissioner of Patents and Trademarks
The United States Patent and Trademark Office
Washington, D.C. 20231

DECLARATION OF DR. DEAN L. ENGELHARDT IN SUPPORT OF ADEQUATE DESCRIPTION AND ENABLEMENT

I, Dean L. Engelhardt, hereby declare as follows:

1. I am the Dean L. Engelhardt who is named as an applicant on the above-identified application. I am a co-inventor of the subject matter claimed in this application. Furthermore, I am familiar with the contents of this application.

2. I am currently employed by Enzo Biochem, Inc., 527 Madison Avenue, New York, New York 10022 as Senior Vice President, having held that position since 1988. Prior to my employment at Enzo Biochem, Inc., I was Associate Professor of Microbiology at Columbia University College of Physicians and Surgeons, New York City, having earlier obtained my doctorate from Rockefeller University in New York City.

3. In addition to my position as Senior Vice President of Enzo Biochem, Inc., I have also served as Director of Research in which capacity I have overseen scientific research activities for the company and its subsidiaries. I also continue to oversee various research projects. Among my responsibilities at Enzo Biochem, Inc. have been the development of new nucleic acid technology and hybridization formats, including new diagnostic and therapeutic approaches and agents based upon nucleic acid technology.

4. I understand that the presently pending claims in this application are directed to a nucleotide in which a moiety Sig is covalently attached to the phosphate moiety (a di-phosphate or tri-phosphate) directly or via a chemical linkage. Sig is capable of non-radioactive detection when attached to the phosphate or when the nucleotide is incorporated into an oligo- or polynucleotide or other composition. I further understand that other presently pending claims are directed to an oligo- or polynucleotide and to other compositions including those comprising a polymeric compound - all of which comprise at least one nucleotide in which a moiety Sig capable of non-radioactive detection is attached to the phosphate moiety thereof directly or via a chemical linkage.

5. In further detail, I understand that the presently pending claims are directed to the just-described nucleotide (278-301, 308-309, 373-394 and 401-404), the oligo- or polynucleotide (310-337 and 405-432), and other compositions (303-307, 338-372 and 433-453) comprising phosphate-modified nucleotides in which a moiety Sig capable of non-radioactive detection is attached thereto directly or via a chemical linkage.

A. Claim 278 is independent and defines the nucleotide as having the formula Sig - PM - SM - BASE wherein PM is selected from a di-phosphate or a tri-phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from a pyrimidine, a purine and a deazapurine, or analog thereof. PM is attached to SM and BASE is attached to SM. Sig is covalently attached to PM directly or via a chemical linkage and it represents a moiety capable of non-radioactive detection when attached to PM. Furthermore, the claimed nucleotide is defined as being capable of incorporation into an oligo- or polynucleotide. Other embodiments of the aforementioned nucleotide include those defining the self-signaling or self-indicating or self-detecting nature of Sig (claim 279); the Sig moiety comprising at least three carbon atoms (claim 280); the

covalent attachment or chemical linkage of Sig to PM (claims 281-287); specific members of Sig (claim 288-301); and the nucleotide comprising a deoxyribonucleotide (claim 308) and a ribonucleotide (claim 309).

B. I also understand that the presently pending claims define an oligo- or polynucleotide comprising at least one such phosphate-modified nucleotide, the oligo- or polynucleotide being terminally ligated or attached to a polypeptide (claim 302). The claims also include other compositions comprising an oligo- or polynucleotide including at least one such phosphate-modified nucleotide and a polypeptide capable of forming a complex with Sig and a moiety which can be detected when the complex is formed (claims 303-307). My understanding of the present claims extend to the oligo- or polynucleotide of which claim 310 is independent and arguably the broadest. Claim 310 defines the oligo- or polynucleotide as comprising at least one nucleotide having the formula

Sig - PM - SM - BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from a pyrimidine, a purine and a deazapurine, or analog thereof. PM is attached to SM and BASE is attached to SM. Sig is covalently attached to PM directly or via a chemical linkage and represents a moiety capable of non-radioactive detection when attached to PM or when the nucleotide is incorporated into the oligo- or polynucleotide. Other dependent claims directed to this oligo- or polynucleotide include embodiments of the self-signaling or self-indicating or self-detecting nature of Sig (claim 311); Sig as comprising at least three carbon atoms (claim 312); the covalent attachment or chemical linkage of Sig to PM (claim 313-320); specific members of Sig (claims 321-334); the attachment of Sig to a terminal nucleotide (claims 335-337); and the nucleotide comprising a deoxyribonucleotide (claim 427) and a ribonucleotide (claim 428).

C. My understanding of the presently pending claims also extend to the composition comprising a polymeric compound having attached directly or indirectly thereto at least one nucleotide having the formula:

Sig - PM - SM - BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from a pyrimidine, purine and a deazapurine, or analog thereof. PM is attached to SM, BASE is attached to SM, and Sig is covalently attached to PM directly or via a chemical linkage and it represents a moiety capable of non-

radioactive detection when attached to PM and when the nucleotide is incorporated into such composition. Claim 338 is independent and is arguably the broadest such composition. Other dependent claims are directed to embodiments describing the self-signaling or self-indicating or self-detecting nature of Sig (claim 339); Sig as comprising at least three carbon atoms (claim 340); the covalent attachment or chemical linkage of Sig to PM (claims 341-348); specific members of Sig (claims 349-361); the composition and a complex-forming polypeptide (claims 363-368); and specific members of the polymeric component (claims 369-372).

D. I also understand that other claims are pending in which the aforementioned moieties (BASE, PM and Sig) are attached to a pentose ring sugar moiety (SM) given by a structural formula set forth in these other claims. The claims reciting such a structural formula include those that are directed to a phosphate-modified (a di-phosphate or tri-phosphate) nucleotide (claims 373-394 and 401-404); an oligo- or polynucleotide or other composition comprising at least one such nucleotide (claims 395-400); an oligo- or polynucleotide comprising at least one phosphate-modified nucleotide (claims 405-432); and a composition comprising a polymeric compound attached directly or indirectly to at least one such phosphate-modified nucleotide (claims 433-453).

6. I have read the two Office Actions dated June 20, 1996 and June 25, 1997 that were issued in connection with this application. I understand that in both Office Actions the specification of this application was objected to and the claims were rejected for lack of adequate description and enablement.

A. The Examiner's position on the description issue taken from the June 20, 1996 Office Action is as follows:

> Claims 207-224 and 227-262 are drawn to nucleotides having the "Sig" moiety attached to the phosphate moiety wherein the Sig moiety is limited to one of several molecular classes such as "at least three carbon atoms, a glycosidic linkage moiety, biotin, iminobiotin, ferritin, an antigen, a hapten, an antibody, etc.
>
> Support for these claims was pointed out in original claims 125, 41, 84, 126, 129, 127 and 128. However, these claims are drawn to nucleotides in which the "Sig" moiety is attached to the base.
>
> The only support that was found in the original disclosure was in a passage on pages 96-97 which begins "By way of summary." This passage defines "Sig" as binding to either base, sugar or phosphate and then defines "Sig" to include the particular products in

the newly presented claims. However, there is no explicit description of the various claimed products bound to the phosphate anywhere in the specification. In contract, the base-linked "Sig" moieties have numerous complex chemical reactions which are necessary to synthesis the various products. These reactions include various solvents, reactants and protecting groups which are necessary so that only the base was modified and not the reactive groups on the sugar or phosphates. Thus, an explicit description of the "phosphate-Sig" reactions would have been expected in order for a skilled artisan to have reasonably concluded that the original disclosure evidenced "possession" of the currently claimed invention.

Thus, in view of the phrase "by way of summary" and the absence of any "phosphate-Sig" reactions to summarize; and in view of the complex nature of these reactions, the skilled artisan would not have reasonably expected this specification to put the artisan in possession of the invention as now claimed.

Since support for these claims was not found where pointed out nor elsewhere in the specification, these claims are considered "new matter."

B. The Examiner's position on enablement as set forth in the June 20, 1996 Office Action is as follows:

Claims 204-224 and 227-262 are broadly drawn to nucleotides having various "Sig moieties" attached to the phosphate moiety.

The specification contains a sufficiently detailed disclosure, such as in Examples I-VII, to enable the construction of "sig-base" nucleotides, that is nucleotides in which the "Sig" moiety is linked to the base. It is noted that these reactions contain many specific solvents, reactants and protecting groups. This detailed disclosure enables one to obtain a reasonable product yield, a product of suitable stability for it's intended use in nucleic acid detection assays and a product reasonably free of unwanted side products in which the Sig moiety is attached at the wrong places on the nucleotide.

However, there is no analogous disclosure for the attachment of the "Sig-phosphate" nucleotides. The broadly claimed "Sig moieties" include a very diverse population of molecules, from simple inorganic compounds like radioactive cobalt to the complex organic molecules like enzymes. Accordingly, there are a vast number of possible chemical reaction schemes one could attempt. Without specific guidance or examples, the skilled artisan would expect that the vast majority of these reaction schemes would fail. Either the product yields would be low, the products would be too unstable or the products would be too hard to purify away from extraneous side products.

It is difficult to predict the behavior of a complex organic molecule with numerous functional groups: primary amines, carboxyl groups and alcohol groups. There is no way to establish, before the fact, which reaction conditions will result in high yields and stable products that can be purified from extraneous byproducts.

The level of skill is high in this field. Nevertheless, in view of the large scope of these claims, the lack of any guidance or specific

examples, the high degree of unpredictability, the complex nature of the invention which requires both inorganic and organic chemical syntheses; it would have required undue experimentation to enable a reasonable number of embodiments within the scope of these claims.

C. I also understand that the enablement issue was maintained by the Examiner in the most recent June 25, 1997 Office Action, the Examiner stating there:

> Applicant argues that Example V, citing Halloran et al., describes labeling of the phosphate moiety with "Sig." This argument is not persuasive for two reasons.
>
> First, essential subject matter cannot be incorporated by reference to a research article. Second, the claims are not limited to a carbodiimide mediated linking of proteins to nucleotides but read generally on any "Sig" linked to the phosphate moiety by any method. Thus, the scope of the described subject matter is very different from the scope of the claimed subject matter. This difference in scope is reflected in the response filed 12/20/96, page 6. first paragraph: "[I]t is evident that at least one means of coupling nucleotide and oligonucleotides to labels through the phosphate moiety was available . . ." Since support for the subject matter of the same scope was not found, nor was it pointed out, the rejection under 35 U.S.C. §112, first paragraph, description requirement is MAINTAINED.
>
> Applicant argues that the claims of US Pat. 5,260,433 is evidence of descriptive support and enablement for the present claims. This argument is not persuasive. Each case is argued on it's own merits. Any arguments made in other cases must be made of record in this case in order to be considered.

7. It is my opinion that the originally filed specification does indeed support the subject matter of the pending claims which are adequately described to the point that a skilled artisan would have reasonably concluded that the original disclosure evidenced possession of the invention currently being claimed. It is also my opinion that the specification provides a disclosure sufficiently enabling so that the skilled artisan, armed with the disclosure and knowledge in the art at the time the application was originally filed in 1982, would have been able to practice the claimed invention without excessive experimentation, or to put it in other words, to practice the invention with a minimum of experimentation. I am making this Declaration to substantiate both the support and adequate description in the specification for the claims and the enabling nature of the specification.

8. A. With respect to the support and description in the specification for the presently claimed invention, I offer the following remarks. In order to understand the basis of this invention, it would be helpful to describe briefly the state of

technology with respect to nucleic acid labeling and detection in the early 1980s. In 1981, Dr. David C. Ward and his group at Yale became textbook celebs for their discovery that nucleotides could be non-radioactively labeled in the so-called non-disruptive positions of the base without substantially interfering with the capability of the labeled nucleotide to be incorporated into an oligo- or polynucleotide, and without substantially interfering with the capability of the oligo- or polynucleotide to be detected by means of the labeled nucleotide that was incorporated. Prior to 1981, nucleic acids were conventionally labeled with radioactive isotopes, most notably ^{32}P. With Dr. Ward's discovery, the world turned en masse to non-radioactive labeling of nucleic acids, that discovery culminating in the issuance of several United States and foreign patents including the following: U.S. Patent Nos. 4,711,955; 5,328,824; 5,449,767; 5,476,928; and European Patent Nos. 0 063 879 B1 and 0 329 198 A2. The latter is an allowed application that has not yet been formally granted.

B. The principles or criteria behind the Ward discovery are exquisitely set forth in their patent specifications. In U.S. Patent No. 5,328,824, for example, the Ward "criteria" for base labeling nucleotides are described in columns 6 and 7 under the section titled "DETAILED DESCRIPTION OF THE INVENTION:'

> Several essential criteria must be satisfied in order for a modified nucleotide to be generally suitable as a substitute for a radioactively-labeled form of a naturally occurring nucleotide. First, the modified compound must contain a substituent or probe that is unique, i.e., not normally found associated with nucleotides or polynucleotides. Second, the probe must react specifically with chemical or biological reagents to provide a sensitive detection system. Third, the analogs must be relatively efficient substrates for commonly studied nucleic acid enzymes, since numerous practical applications require that the analog be enzymatically metabolized, e.g., the analogs must function as substrates for nucleic acid polymerases. For this purpose, probe moieties should not be placed on ring positions that sterically, or otherwise, interfere with the normal Watson-Crick hydrogen bonding potential of the bases. Otherwise, the substituents will yield compounds that are inactive as polymerase substrates. Substitution at ring positions that alter the normal "anti" nucleoside conformation also must be avoided since such conformational changes usually render nucleotide derivatives unacceptable as polymerase substrates. Normally, such considerations limit substitution positions to the 5-position of a pyrimidine and the 7-position of a purine or a 7-deazapurine.
>
> Fourth, the det ction system should be capable of interacting with prob substituents incorporated into both single-stranded and double-stranded polynucleotides in order to be compatible with nucleic

acid hybridization methodologies. To satisfy this criterion, it is preferable that the probe moiety be attached to the purine or pyrimidine through a chemical linkage or "linker arm" so that it can readily interact with antibodies, other detector proteins, or chemical reagents.

Fifth, the physical and biochemical properties of polynucleotides containing small numbers of probe substituents should not be significantly altered so that current procedures using radioactive hybridization probes need not be extensively modified. This criterion must be satisfied whether the probe is introduced by enzymatic or direct chemical means.

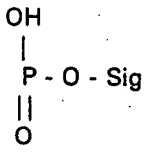
Finally, the linkage that attaches the probe moiety should withstand all experimental conditions to which normal nucleotides and polynucleotides are routinely subjected, e.g., extended hybridization times at elevated temperatures, phenol and organic solvent extraction, electrophoresis, etc.

All of these criteria are satisfied by the modified nucleotides described herein.

9. A. A short time after Ward's discovery, it was unexpectedly discovered by the instant inventors - all of whom were scientists at Enzo - that nucleic acid labeling and detection could be extended far beyond, but moreover, in total contradiction to Ward's discovery and criteria. Our subsequent and unexpected discovery that culminated in the filing of the first application in the family in 1982 flew headlong against Ward because the positions for labeling the nucleic acid now involved the so-called "disruptive" and "semi-disruptive" positions in the base. Moreover, the novel labeling and labeled compositions involved not only such positions in the base moiety, but the sugar and phosphate moieties as well. This discovery with respect to the phosphate moiety is set forth in several portions in the instant specification. In the specification, page 94, last paragraph, and continuing through page 95, first paragraph, the phosphate-modified nucleotides and compositions of the present invention are specifically disclosed but not for the first time:

> Still further, nucleotides in accordance with the practices of this invention include the nucleotides having the formula,
>
> Sig
> |
> P - S - B
>
> wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety. In these special nucleotides the P moiety is attached to the 3' and/or the 5' position of the S moiety when the nucleotide is deoxyribonucleotide and at the 2', 3' and/or 5' position when the nucleotide is a ribonucleotide. The base B is either a purine or a pyrimidine and the B moiety is attached from the N1 or the N9 position to the 1' position of the sugar moiety when said B moiety is a

pyrimidine or a purine, respectively. The Sig chemical moiety is covalently attached to the phosphoric acid P moiety via the chemical linkage



said Sig, when attached to said P moiety being capable of signalling itself or making itself self-detecting or its presence known and desirably the nucleotide is capable of being incorporated into a double-stranded or DNA, RNA or DNA-RNA hybrid.

Later on page 96, and continuing through the first paragraph on page 98, further description of the present invention is amply provided:

By way of summary, as indicated hereinabove with respect to the make-up of the various special nucleotides in accordance with this invention, the special nucleotides can be described as comprising a phosphoric acid moiety P, a sugar moiety S and a base moiety B, a purine or pyrimidine, which combination of P-S-B is well known with respect to and defines nucleotides, both deoxyribinucleotides and ribonucleotides. The nucleotides are then modified in accordance with the practices of this invention by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig. The chemical moiety Sig so attached to the nucleotide P-S-B is capable of rendering or making the resulting nucleotide, now comprising P-S-B with the Sig moiety being attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide, especially a double-stranded polynucleotide, such as double-stranded DNA, a double-stranded RNA or a double-stranded DNA-RNA hybrid. The Sig moiety desirably should not interfere with the capability of the nucleotide to form a double-stranded polynucleotide containing the special Sig-containing nucleotide in accordance with this invention and, when so incorporated therein, the Sig-containing nucleotide is capable of detection, localization or observation.

The Sig moiety employed in the make-up of the special nucleotides of this invention could comprise an enzyme or enzymic material, such as alkaline phosphatase, glucose oxidase, horseradish peroxidase or ribonuclease. The Sig moiety could also contain a fluorescing component, such as fluorescein or rhodamine or dansyl. If desired, the Sig moiety could include a magnetic component associated or attached thereto, such as a magnetic oxide or magnetic iron oxide, which would make the nucleotide or polynucleotide containing such a magnetic-containing Sig moiety detectable by magnetic means. The Sig moiety might also include an electron dense component, such as ferritin, so as to be available by observation. The Sig moiety could also include a radioactive isotope component, such as radioactive cobalt, making the resulting nucleotide observable by radiation detecting means. The Sig moiety could also include a hapten component or per se be capable of complexing with an antibody specific thereto. Most usefully, the Sig moiety is a polysaccharide or

> oligosaccharide or monosaccharide, which is capable of complexing with or being attached to a sugar or polysacchardie binding protein, such as a lectin, e.g. Concanavilin A. The Sig component or moiety of the special nucleotides in accordance with this invention could also include a chemiluminescent component.
>
> As indicated in accordance with the practices of this invention, the Sig component could comprise any chemical moiety which is attachable either directly or through a chemical linkage or linker arm to the nucleotide, such as the base B component therein, or the sugar S component therein, or the phosphoric acid P component thereof.
>
> The Sig component of the nucleotides in accordance with this invention and the nucleotides and polynucleotides incorporating the nucleotides of this invention containing the Sig component are equivalent to and useful for the same purposes as the nucleotides described in the above-identified U.S. patent application Serial No. 255,223. More specifically, the chemical moiety A described in U.S. patent application Serial No. 255,223 is functionally the equivalent of the Sig component or chemical moiety of the special nucleotides of this invention. Accordingly, the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm as described in U.S. patent application Ser. No. 255,223, as indicated by the dotted line connecting B and A of the nucleotides of U.S. Serial No. 255,223. The various linker arms or linkages identified in U.S. Ser. No. 255,223 are applicable to and useful in the preparation of the special nucleotides of this invention.

Even further embodiments of the instant nucleotides and compositions are later described in the specification, on page 103, first full paragraph; and on page 103, last paragraph, continuing through page 106, first paragraph.

B. In all, there are no fewer than nine (9) instances where the Sig moiety component is described in the specification as being attached to the phosphate moiety P, the sugar moiety S and/or the base moiety B! These nine separate and distinct instances include the following:

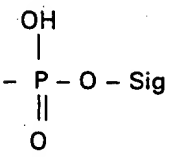
Specification	Description
page 90, last paragraph	. . . and a signalling chemical moiety Sig covalently attached thereto, either to the P, S or B moiety.
page 93, first paragraph	. . . include a chemical moiety Sig covalently attached to the P, S and/or B moieties.
page 96, first paragraph	. . . by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig.

page 98, first paragraph	. . . the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm . . .
page 103, first full paragraph	. . . and the signalling or self-detecting moiety, Sig, covalently attached to either the P, S or B moieties, as indicated hereinabove, . . .
page 104, first paragraph	. . . nucleotides in accordance with this invention containing the above-described components P, S, B and Sig, . . .
page 105, first paragraph	. . . the nucleotides of this invention include the P, S, B and Sig components wherein the Sig is covalently attached to either the P, S or B moieties
page 105, second paragraph	The moiety Sig attached to the special nucleotides of this invention containing the other moieties or components P, S, B provides a site per se for the attachment thereto, the Sig component, . . .
page 106, first paragraph	. . . the special P, S, B and Sig-containing nucleotides of this invention, . . .

C. In addition to those portions in the specification cited above, Example V describes a method for attaching biotin, one of the embodiments for Sig, to the phosphate moiety of a mononucleotide and an oligonucleotide that are coupled to a protein, poly-L-lysine. Using the procedure in Example V in the specification (page 57), the biotinylated poly-L-lysine is coupled to a terminal oxygen of the phosphate moiety or to a terminal phosphorus. These reaction schemes are set forth in Figure 1 on page 374 in Halloran and Parker, J. Immunol., 96:373 (1966) cited in Example V, page 57 in the specification (a copy of Halloran's publication also having been attached hereto as Exhibit 1).

D. In my opinion, the originally filed claims are a telling piece of evidence with respect to using any of the embodiments of Sig for either the base, sugar or phosphate moieties. Here, one need only look at original claim 143 that recites:

> A nucleotide having the general formula P–S–B wherein P is the phosphoric acid moiety, S the sugar or monosaccharide moiety and B the base moiety, said nucleotide having covalently attached to the P or S or B moiety a chemical moiety Sig, said Sig chemical moiety when attached to the P moiety is attached thereto via the chemical linkage,



and when Sig is attached to the S moiety, the S moiety is a ribose group, said chemical moiety Sig when attached to said P, S or B being capable of signalling itself or makes itself self-detecting or its presence known.

It is clear from the language of original claim 143 that Sig could be attached to the phosphate (or phosphoric acid), sugar and base moieties in accordance with this invention.

E. The chemical reactions by which substituents are attached to the oxygen or the phosporus atoms of a phosphate or phosphoric acid moiety in a nucleotide (or an oligo- or polynucleotide or other polymer such as a protein) were known in the art prior to the first filing of this application in 1982. Illustrative of the reactions and chemistry known in the art before 1982 are those summarized below.

Reactions involving the oxygen

Goody et al., JACS 93:6252-6257 (1971) [Exhibit 2] disclose a reaction for adding a diphenyl to the oxygen atoms of nucleoside di- and triphosphates. See, for example, Scheme I on page 6253.

Eckstein et al., Biochemistry 14:5225-5232 (1975) [Exhibit 3] disclose guanosine 5'-di- and triphosphate derivatives with modified terminal phosphates in which the following substituents are added to the terminal oxygen: methyl, aminoethyl, acetylaminoethyl and phenyl. See, for example, the discussion under "*Synthesis of Analogues*" beginning on page 5226, left column, and continuing through page 5228, right column. See also Figure 1 on page 5226.

Armstrong et al., European Journal of Biochemistry 70:33-38 (1976) [Exhibit 4] disclose ATP and UTP analogues modified in the phosphate moieties in which a methyl or a phenyl group is attached to a terminal oxygen. See structures I b) and I c) on page 33, right column.

Reactions involving the phosphorus

Miller et al., Biochemistry 18:5134-5143 (1979) [Exhibit 5] disclose a series of dideoxyribonucleoside methylphosphonate analogues in which a methylene group is contained in the internucleoside linkage. See, for example, the discussion under "*Preparation of Dinucleoside Methylphosphonates*" beginning on page 5136, right column, and continuing through page 5137.

Miller et al., Biochemistry 20:1874-1880 (1981) [Exhibit 6] disclose the preparation of oligodeoxyribonucleoside methylphosphonates. See, for example, the discussion under "*Preparation of Oligonucleoside Methylphosphonates*" beginning on page 1875, left column, and continuing through the first four lines of the right column. See also Table I at the top of page 1876.

Beaucage et al., Tetrahedron Letters 22:1859-1862 (1981) [Exhibit 7] disclose deoxynucleoside phosphoramidites depicted in structures Ia-d and IIIa-d on page 1859 and structures Ia, II and IIIa on page 1861.

Miyoshi et al., Nucleic Acids Research 8:5491-5505 (1980) [Exhibit 8] disclose the preparation of three oligonucleotides, i.e., hexadecanucleotides in which di- and trinucleotides are used as incoming 3'-phosphodiester units. See, for example, Figure 1 on page 5493.

Gait et al., Nucleic Acids Research 8:1081-1096 (1980) [Exhibit 9] disclose the preparation of oligodeoxyribonucleotides up to 12 units long using phosphotriesters.

Duckworth et al., Nucleic Acids Research 9:1691-1706 (1981) [Exhibit 10] disclose the preparation of heptadecadeoxyribonucleotides using phosphotriesters.

Ohtsuka et al., Tetrahedron Letters 23:3081-3084 (1982) [Exhibit 11] disclose the synthesis of dodecadeoxynucleotides using phosphotriesters.

Gough et al., Tetrahedron Letters 22:4177-4180 (1981) [Exhibit 12] disclose the construction of oligodeoxyribonucleotides using phosphotriesters.

In addition to the coupling reactions disclosed in Halloran et al. cited on page 57 in the instant specification (copy attached as Exhibit 1), other procedures were known in the art prior to 1982 for coupling nucleic acid sequences to other biological polymers, including protein and polysaccharides. Among the coupling reactions known before 1982 are those listed below.

Reactions for coupling nucleic acids to other polymers (e.g., proteins, polysaccharides)

Brutlag et al., Biochemistry 8:3214-3218 (1969) [Exhibit 13] disclose cross-linking deoxyribonucleic acid to histone in nucleohistone using formaldehyde.

Manning et al., Chromosoma 53:107-117 (1975) [Exhibit 14] disclose the attachment of biotin to *Drosophila* rRNA via a cytochrome c bridge.

Politz et al., Biochemistry 20:372-378 (1981) [Exhibit 15] disclose the cross-linking of RNA to protein in *Escherichia coli* 30S ribosomal subunits using a heterobifunctional cross-linking reagent.

Cramer et al., Chemische Berichte 92:384-391 (1959) [Exhibit 16] disclose the attachment of polynucleotide sequences to polysaccharides in which 20 units of the latter was described as preferred.

ADEQUATE DESCRIPTION

10. A. It is my opinion that the above-cited portions in the specification adequately describe the presently claimed invention, including particularly those embodiments for the Sig component set forth in the claims. My opinion extends to those embodiments cited by the Examiner in the June 20, 1996 Office (page 3, first paragraph), specifically those where Sig is a moiety containing at least three carbon atoms (claims 280, 312, 340, 376, 408 and 436); Sig includes a glycosidic linkage moiety (claims 287, 319, 347, 380, 412 and 443); Sig is selected from biotin and iminobiotin (claims 288, 321, 349, 381, 413 and 449); Sig comprises ferritin (claims 289, 322, 350, 382 and 414); and Sig is selected from an antigen,

a hapten and an antibody (claims 288, 321, 349, 381, 413 and 449). It is also my opinion that the specification reasonably conveys the description that Sig may be any of the foregoing when attached to the phosphate moiety in the presently claimed nucleotides and other composition claims comprising the nucleotides because of numerous instances (nine in all!) where Sig is described as being attached to the phosphate moiety P, the sugar moiety S and/or the base moiety B. Original claim 143 is particularly significant, in my opinion, because the language specifically recites "said nucleotide having covalently attached to the P or S or B moiety a chemical moiety Sig . . ." The fact that dependent claims for the various embodiments of Sig were not included with the originally filed claims directed to the phosphate modified nucleotides (claim 141) does not in any way detract from my own conviction and opinion that the support and description for such claims would have been clearly and reasonably conveyed by reading the specification, as described in the portions cited above. It is very clear in my opinion that the specification discloses that the embodiments of Sig are to be applied - without limitation - in the disruptive and semi-disruptive positions of all three moieties recited in the independent claims, i.e., the base, sugar and phosphate moieties.

B. To elaborate further, the claimed products encompassing the various embodiments for Sig being attached to the phosphate moiety are clearly supported by the specification, particularly because all such embodiments for Sig are described as functionally equivalent for purposes of the present invention which is directed to disruptive and semi-disruptive modifications of nucleotides involving the phosphate, sugar and base moieties. The fact that one description of the phosphate-modified nucleotides is found in a paragraph that opens with "By way of summary" is of no import for at least three substantial reasons. First, as discussed above in Paragraph 9B above, there are at least nine separate instances in the specification where the attachment of Sig to any or all of the phosphate, sugar and base moieties is disclosed. The specification reasonably conveys, therefore, that the coinventors were in possession of the instantly claimed embodiments for Sig in the phosphate-modified nucleotides and compositions at the time this application was originally filed in 1982. Second, the paragraph beginning with "By way of summary" itself specifically discloses that "[t]he nucleotides are then modified in accordance with the practices of this inv ntion by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig." That recitation also reasonably conveys that the coinventors were in

possession of the instantly claimed subject matter at the time the application was first filed in 1982 because the specification clearly informs the reader that Sig can be attached to any of the three moieties in the nucleotide - and even to more than one moiety at the same time. Third, in the subsequent three paragraphs (page 96, last paragraph, through page 98, first paragraph) that describe embodiments for Sig, at least two instances occur where Sig is described as being attached to the phosphate, sugar or base moieties of the nucleotide:

> . . . the Sig component could comprise any chemical moiety which is attachable either directly or through a chemical linkage or linker arm to the nucleotide, such as the base B component therein, or the sugar S component therein, or the phosphoric acid P component thereof.
> [page 97, first full paragraph]

> . . . the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm . . .
> [page 98, first paragraph]

One could reasonably conclude from reading the three paragraphs describing the embodiments for Sig that each are applicable to not only the base moiety, but the phosphate moiety (and the sugar moiety) as well.

C. All of the foregoing reasons supports my conclusion that the specification reasonably conveys that the coinventors were in possession of the subject matter now being claimed.

D. As explained above in Paragraphs 9C and 9D, numerous reactions were known in the art for modifying the phosphate moiety of a nucleotide. It is my opinion that the specification as filed originally in 1982 reasonably conveys that the coinventors were in possession of the subject matter now being claimed and that that impression would not and does not require recitation of the litany of "phosphate-Sig" reactions indicated by the Examiner in the June 25, 1996 and June 20, 1997 Office Actions. Phosphate-Sig reactions were known in the art and an explicit description of such known reactions would not have been necessary to convey the impression that the coinventors were in possession of the subject matter now being claimed.

ENABLEMENT

11. A. It is also my opinion that the specification provides an enabling disclosure for all of the pending claims in this application. As discussed above in Paragraph 9C, Example V in the specification (page 57) provides a means for labeling the oxygen or the phosphorus of a nucleotide. As also noted above in Paragraph 9D, the chemistry and reactions for attaching substituents to the oxygen or phosphorus atoms in a nucleotidyl phosphate or phosphoric acid moiety were already known in the art at the time the initial application was filed in 1982. Although listed above after Paragraph 9D, the known chemistry and reactions are listed below for the sake of completeness.

Chemistry/Reaction	Citation	Description
oxygen	Goody et al. (1971) [Exhibit 2]	diphenyl addition to oxygen of nucleoside di- & triphosphates
oxygen	Eckstein (1975) [Exhibit 3]	methyl, aminoethyl, acetylaminoethyl & phenyl added to GDP & GTP
oxygen	Armstrong et al. (1976) [Exhibit 4]	methyl & phenyl attached to ATP & UTP analogs
phosphorus	Miller et al. (1979) [Exhibit 5]	methylene addition to prepare dideoxyribo-nucleoside methylphos-phonates
phosphorus	Miller et al. (1981) [Exhibit 6]	methylene addition to prepare dideoxyribo-nucleoside methylphos-phonates
phosphorus	Beaucage (1981) [Exhibit 7]	preparation of deoxynucleoside phosphoramidites
phosphorus	Miyoshi et al. (1980) [Exhibit 8]	oligonucleotide synthesis using phosphotriesters
phosphorus	Gait et al. (1980) [Exhibit 9]	oligodeoxynucleotide preparation using phosphotriesters

phosphorus	Duckworth et al. (1981) [Exhibit 10]	heptadecadeoxyribonucleotide preparation using phosphotriesters
phosphorus	Ohtsuka et al. (1982) [Exhibit 11]	dodecadeoxynucleotide preparation using phosphotriesters
phosphorus	Gough et al. (1981) [Exhibit 12]	oligodeoxyribonucleotide preparation using phosphotriesters
coupling	Halloran et al. (1966) [Exhibit 1]	coupling biotinylated poly-L-lysine to mono- and oligonucleotides
coupling	Brutlag et al. (1969) [Exhibit 13]	DNA to histone
coupling	Manning et al. (1975) [Exhibit 14]	biotin to rRNA using cytochrome c bridge
coupling	Politz et al. (1981) [Exhibit 15]	RNA to ribosomal protein
coupling	Cramer (1959) [Exhibit 16]	polynucleotide sequences to polysaccharides

B. It is my opinion that the subject matter now being claimed in this application, claims 278-453, could have been practiced in 1982 - with minimal experimentation and not with excessive experimentation - from a reading of the specification, particularly Example V, and further in light of the chemistry and reactions known at that time. The known chemistry and reactions are illustrated by the scientific publications cited in this Declaration above (Exhibits 1-16).

Lastly, although not an issue of enablement or adequate description, I should point out that none of the publications submitted in this Declaration (Exhibits 1-16) disclose or suggest the instantly claimed invention in which a Sig moiety is attached to the phosphate moiety of a nucleotide and which is capable of non-radioactive detection when so attached and further, is capable of incorporation into other compositions, including an oligo- or polynucleotide.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false

statements and th like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nov. 24, 1997
Date

Dean L. Engelhardt
Dean L. Engelhardt

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